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东海赤潮高发区及台湾海峡南部上升流海域好氧不产氧光合异养细菌的生态过程研究

Ecological Research of Aerobic Anoxygenic Phototrophic Bacteria in the Red-tide-frequent-occurrence-area of the East China Sea and in the Upwelling Area of Southern Taiwan Strait

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摘 要

好氧不产氧光合异养细菌（Aerobic anoxygenic phototrophic bacteria，AAPB）是一组新的能进行光合营养的异养细菌，它在碳和其它生源要素的生物地球化学循环中扮演着独特而重要的角色，揭示其在某些特殊的海洋生态系统中的生态过程和响应机制具有非常重要的意义。本论文在赤潮高发的 2005 年 3-6 月及 2006 年 4-5 月对东海赤潮高发区开展了 AAPB 分子生态学研究，此外还于 2005 年冬季、2005 年夏季和 2006 年夏季研究了台湾海峡南部上升流海域 AAPB 的时空分布及其对上升流事件的响应。

从 2005-2006 年东海赤潮高发区的所有航次综合来看，在本论文已知的因子当中，赤潮藻种类、光照、叶绿素 *a* 和温度是调控 AAPB 分布的主要因子，不同因子在不同航次当中调控作用的重要性有所不同。总体来讲 2005 年和 2006 年年际变化不明显。从表中底三个水层随着航次的变化综合来看，各项生物因子都是春季要明显低于夏季，营养盐则是春季明显高于夏季；当叶绿素 *a* 浓度相对较低的时候，AAPB、总异养细菌及 AAPB%与叶绿素 *a* 的走势大体一致，而当叶绿素 *a* 浓度很高即发生赤潮的时候，则很不一样，此时发生赤潮的优势藻种类起了重要的调控作用。AAPB、总异养细菌及 AAPB%与温度有着相似的变化趋势，与无机营养盐（磷酸盐和硝酸盐）有着大致相反的变化趋势（这与该研究为赤潮高发的特殊时期有关），与盐度、透明度的关系不明显。对 2005-2006 年所有航次的各个因子进行相关性分析发现，AAPB 和总异养细菌的分布趋势相似；AAPB、总异养细菌及 AAPB%与温度极显著正相关。

2005 年东海赤潮高发区 AAPB 对赤潮响应的研究发现，总异养细菌丰度在赤潮站位比非赤潮站位要高或者相近。而 AAPB 对赤潮的响应显得更加复杂和多样化，即在发生中肋骨条藻和旋链海链藻双相赤潮、东海原甲藻和米氏凯伦藻双相赤潮或夜光藻赤潮时 AAPB 丰度比同时期的其它未发生赤潮的站位要高；当东海原甲藻或米氏凯伦藻单独发生赤潮，或者赤潮消亡时，AAPB 丰度没有显著的变化；但在血红哈卡藻发生赤潮时，AAPB 丰度明显降低，仅占非赤潮站位平均值的 20%左右。除了东海原甲藻和米氏凯伦藻双相赤潮外，AAPB%对赤潮

的响应与 AAPB 基本一致。可见在东海赤潮高发区，AAPB 对赤潮的响应依赖于赤潮优势藻种的变化，且在一定程度上与叶绿素 *a* 的浓度不相关。

我们通过 PCR 扩增 *pufLM* 基因的方法，首次研究了东海赤潮高发区赤潮发生过程中 AAPB 遗传多样性的变化，同时比较了 *pufL* 和 *pufM* 两段序列在分析 AAPB 遗传多样性中的差异。结果表明在赤潮高发的春夏季时该研究海域的所有 AAPB 都是属于 α 亚纲的，且绝大部分为 *Roseobacter*-like 类群。在赤潮发展过程中，AAPB 的主要优势类群发生了一系列变化，表明赤潮的发生及其优势种的演替会显著影响 AAPB 优势类群的分布及更替。除了赤潮发生的状态，营养盐和盐度等环境因子也是影响 AAPB 多样性分布的重要因子。另外，*pufL* 和 *pufM* 两段序列系统发育分析的结果基本上是一致的，但也有一些细微的差异存在。

对台湾海峡南部上升流海域 2005 年冬夏季季节差异的研究发现，2005 年冬季水体混合剧烈，A、B 两个断面的温盐等值线均呈铅直方向，这个季节并未发现深层水涌升现象；2005 年夏季水体层化现象明显，同时受沿岸上升流和浅滩上升流的影响。冬季水体混合使得底层的营养盐被带到表层，此外浙闽沿岸流也为台湾海峡带来了丰富的营养盐，使得冬季营养盐浓度都较高；而夏季因表底层温度差别较大而海水层化现象明显，底层的营养盐无法输送到表层，所以夏季的营养盐浓度会偏低。冬季的叶绿素 *a* 浓度、AAPB 丰度和 AAPB%均显著低于夏季，这可能是温度差异所导致的，但总异养细菌在冬夏季间无显著差异发现。此外，2005 年冬夏两季的叶绿素 *a* 浓度、AAPB 丰度和 AAPB%基本上都是近岸高、离岸低，细菌的分布则没有这种明显的差异，表明 AAPB 相比于其它异养细菌（non-AAPB）与叶绿素 *a* 关系更为密切。

对台湾海峡南部上升流海域 2005 年夏季和 2006 年夏季的年际差异的研究发现，从表层温盐来看，近岸上升流在 2005 年夏季似乎要强于 2006 年夏季，而这种温度和盐度的差异可能是由两个年份间整体的温盐差异所导致的；但浅滩上升流的强度却在 2006 年夏季明显较强；此外 2005 年夏季冲淡水强度要强于 2006 年夏季。生物因子因为受到各种因素的共同调控，所以它们在两个年份间的分布模式不完全一致，其中总异养细菌在两个年份间的分布差异相对叶绿素 *a*、AAPB 和 AAPB%的分布差异要大。

我们的研究还发现，上升流所引发的其它生物和非生物环境的变化对 AAPB 和总异养细菌起着重要的调控作用。在上升流的新涌升阶段，AAPB 和总异养细

菌丰度较低；随着上升流的发展两者丰度均增加并在上升流的成熟期达到最高值；而随着上升流的衰退，AAPB 和总异养细菌丰度开始下降。叶绿素 *a* 在 AAPB 对上升流发展的响应过程当中可能起着更为直接和重要的作用，而营养盐则可能通过一些间接的方式如对叶绿素 *a* 的调控而影响 AAPB 的分布。与 AAPB 不一致的是，总异养细菌与叶绿素 *a* 的相关性不明显，而营养盐则可能直接在总异养细菌对上升流的响应中起着重要的作用。

关键词：好氧不产氧光合异养细菌；东海；赤潮高发区；台湾海峡；上升流；*pufLM* 基因

Abstract

Aerobic anoxygenic phototrophic bacteria (AAPB) are a new functional group of heterotrophic bacteria capable of phototrophy. They play unique and important roles in the biogeochemical cycle of carbon and other elements. Thus, it is very significative to explore the ecological process and response mechanisms of AAPB in some special marine ecological systems. In the present study, molecular ecology of AAPB in the red-tide-frequent-occurrence-area of the East China Sea was investigated during March-June 2005 and April-May 2006. Additionally, we examined the ecological distribution of AAPB in the upwelling region in Southern Taiwan Strait during winter of 2005, and summers of 2005 and 2006, as well as its response to the upwelling development.

Taking together all the cruises in the red-tide-frequent-occurrence-area of the East China Sea during 2005-2006, dominating phytoplankton species, illumination, chlorophyll *a* concentration and temperature were the key factors controlling AAPB distribution, and they played different importance in different cruises. Generally, insignificant variation was found between 2005 and 2006. All the studied biological factors were higher in summer than in spring, except that nutrients had the opposite variance trend. When chlorophyll *a* concentration was low, AAPB, total heterotrophic bacteria and AAPB% basically followed the same trend with chlorophyll *a*. However, the relationship disappeared and the dominating phytoplankton species showed a crucial role when chlorophyll *a* concentration was high, i.e. when blooms happened. AAPB, total heterotrophic bacteria and AAPB% displayed a similar trend with temperature, but they had an opposite tendency with inorganic nutrients, e.g. phosphate and nitrate (The opposite relationship between AAPB and inorganic nutrients was related with the unique period of red tide in this study). Additionally, they showed little correlation with salinity and transparency. In the analysis for the correlation among the various factors in all the cruises of 2005-2006, AAPB showed a similar tendency with total heterotrophic bacteria. Meanwhile, AAPB, total heterotrophic bacteria and AAPB% had an extremely significant and positive relationship with temperature.

During studying the response of AAPB to red tide in the red-tide-frequent-occurrence-area of the East China Sea in 2005, the results showed that total heterotrophic bacterial abundances at the bloom stations were higher than or similar to those at the non-bloom stations during the same time period. Interestingly, the responses of AAPB to red tide seemed to be more diverse and complex. AAPB abundance was higher at the stations with red tide where *Thalassiosira curviseriata* and *Skeletonema costatum*, *Noctiluca scintillans*, or *Prorocentrum donghaiense* and *Karenia mikimotoi* co-dominated than those at the non-bloom stations during the same time period. Little change was observed in *P. donghaiense* or *K. mikimotoi* bloom, or in the decline of bloom. However at the stations with a bloom of *Akashiwo sanguinea*, AAPB abundance only accounted for ~20% of the average abundance of AAPB at the non-bloom stations. In addition, variations of AAPB's proportion to total bacterial abundance (AAPB%) in response to red tide basically followed AAPB abundance, except in the case of *P. donghaiense* and *K. mikimotoi* co-dominating bloom. Overall, the responses of AAPB to red tide are dominating phytoplankton species specific and somewhat independent of chlorophyll *a* concentration.

For the first time we investigated the genetic diversity of AAPB during the whole phytoplankton bloom time course in red-tide-frequent-occurrence-area of the East China Sea by retrieving *pufLM* sequence via PCR, and we also evaluated the difference between *pufL* and *pufM* sequences in analyzing AAPB genetic diversity. According to the phylogenetic tree analysis, all AAPB were related to α-proteobacteria and most of them were considered to be *Roseobacter*-like clade. During the development of red tide, a series of changes happened to the dominating population of AAPB, highlighting that the development of red tide, as wells the succession of the dominating phytoplankton could significantly affect the dynamics of the dominating group in AAPB. Beside, nutrient and salinity were supposed to be important factors in controlling AAPB diversity. In addition, the phylogenetic analysis concerning *pufL* and *pufM* sequences were basically the same, in spite of some tiny variance between them.

Based on the seasonal variation study between winter and summer of 2005 in the upwelling region in Souther Taiwan Strait, a severe water mixing happened in the winter of 2005, with both A and B sections showing a vertical temperature and salinity isoline. Meanwhile, no upwelling was observed in this season. While in the summer of 2005, there was an obvious water stratification phenomenon, and it was affected by the coastal and shallow upwellings. In winter, nutrients were brought to the surface layer from bottom due to water mixing. Additionally, the coastal current from Zhejiang and Fujian provinces also brought abundant nutrients to the Taiwan Strait. Consequently, a relatively high concentration of nutrients was observed in winter. However, due to the water stratification caused by a remarkable temperature gap between surface and bottom layers, nutrients from bottom could not be transferred to the surface layer, with a concomitantly low nutrient concentration in summer. There were higher values of chlorophyll *a* concentration, AAPB abundance and AAPB% in summer than in winter, which might be caused by a temperature discrepancy in these two seasons, while little difference was observed for total heterotrophic bacterial abundance. In addition, both in winter and summer, higher values of chlorophyll *a* concentration, AAPB abundance and AAPB% were basically found inshore than offshore, however, with bacterial abundance showing insignificant variance, indicating that AAPB were more related to chlorophyll *a* than non-AAPB.

For the annual variations between summers of 2005 and 2006 in Southern Taiwan Strait, the coastal upwelling current seemed to be stronger in 2005 than in 2006 due to the lower temperature and higher salinity in the surface water in 2005. However, this difference was probably caused by the whole discrepancy in temperature and salinity between these two years. A stronger shallow upwelling current was observed in summer of 2006, however, with a stronger diluted water in summer of 2005. The biological components showed a different mode between 2005 and 2006, considering that they were co-regulated by many variant factors. A larger difference was found for total heterotrophic bacteria between 2005 and 2006, compared with chlorophyll *a* concentration, AAPB abundance and AAPB%.

Also, the study showed that the change of other biotic and abiotic parameters induced by the upwelling events played important roles in controlling AAPB and total heterotrophic bacteria abundances. In the newly upwelling phase, AAPB and total heterotrophic bacterial abundances were low. Then they increased with the development of the upwelling event, and peaked at its mature phase. However, they began to decrease following the aged upwelling. It is presumed that chlorophyll *a* played a more direct and important role in the response of AAPB to the upwelling events. However, an indirect means might be used by nutrients to affect AAPB distribution, e.g. via regulating chlorophyll *a* concentration. In contrast to AAPB, total heterotrophic bacteria displayed a negligible correlation with chlorophyll *a*. Nutrients might be considered to be important in controlling the response of total heterotrophic